REMARKS

Claims 3-5 and 19-21 currently appear in this application. The Office Action of April 9, 2002, has been carefully studied. These claims define novel and unobvious subject matter under Sections 102 and 103 of 35 U.S.C., and therefore should be allowed. Applicants respectfully request favorable reconsideration, entry of the present amendment, and formal allowance of the claims.

Rejections under 35 U.S.C. 112

Claims 3 and 5 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for *Stachybotrys chartarum*, is said not to reasonably provide enablement for other fungal species.

This rejection is respectfully traversed. Submitted herewith is the declaration of Stephen Vesper, one of the inventors of the subject application, which illustrates the ease with which one skilled in the art can screen for production of hemolysin. This procedure is simple, inexpensive, and fast, and requires no undue experimentation.

Once one has obtained a fungus that produces a hemolysin, one grows the fungus in TSB as taught in the present specification, and one collects the supernatant. This is important because the homogenate, such as used

for asp-hemolysin, introduces a huge number of other fungal proteins, whereas the supernatant is relatively free of most other fungal proteins and makes the purification much easier, since the secreted form of the hemolysin is the active form. Then the hemolysin is purified using standard protein purification procedures described in the present application: Step 1, size fractionation centrifugation; Step 2, ion chromatography; Step 3, gel filtration. Of course, there may be slight variations in buffers, salt concentration, gel matrices, etc., but these are accepted variations that any protein biochemist expects.

With respect to isolating hemolysin from any number of fungi other than *Stachybotrys chartarum,* to demonstrate how easy it is to isolate other fungal hemolysins using the process described in the present application, the inventor isolated the hemolysin from *Penicillium chrsogenum* using the same steps, namely, growth of culture in tryptic soy broth (TSB), size fractionation, centrifugation of supernatant, ion chromatography, and gel filtration, as shown in the VESPER declaration.

Claims 19-21 are rejected under 35 U.S.C. 112, first paragraph, because the specification is said to be enabling only for a method for determining if a mammal

has been exposed to *Stachybotrys chartarum*, and not for other hemolysin-producing fungal species.

As noted above, the declaration of Stephen Vesper submitted herewith makes it clear that once one has determined that a mammal has been exposed to one type of hemolysin producing fungus, one skilled in the art can determine if a mammal has been exposed to any type of hemolysin producing fungus without undue experimentation.

As described in the specification at page 10, paragraph 0039, one skilled in the art can readily determine which fragment of a protein is biologically active, using standard techniques such as those described in the VESPER declaration. Paragraph 0040 of the specification as filed defines an immunoreactive fragment as an amino acid sequence of at least approximately five consecutive amino acids derived from a protein's amino acid sequence, which has the activity needed for the particular test described herein. This is described in more detail in the specification in paragraph 0044. This method of determining if a fragment, derivative, etc. has the proper immunogenic activity requires no undue experimentation, as it uses conventional laboratory techniques which are familiar to one skilled in this art.

The specification in the following paragraphs provides guidance with respect to fragments, derivatives,

etc., that can be used to provide some additional property, such as solubility, which makes the proteins easier to use in the assays.

There is no reason to describe an amino acid sequence for the claimed protein, because there is no claimed protein. The claims are directed to a method for determining if a mammal has been exposed to a hemolysin-producing fungus. The actual structure of the hemolysin is immaterial, and there is thus no need to provide any more than methods for isolating the protein from samples to be tested.

The claimed invention is directed to a method for determining if a mammal has been exposed to a hemolysin producing fungus. This method involves extracting the hemolysin or an active fragment thereof from a sample and testing for the hemolysin or fragment thereof, using conventional tests. The structure of the hemolysins is not critical to the invention, and therefore it is not necessary to provide detailed sequences for the proteins assayed.

The claims at issue are limited to a method for determining if a mammal has been exposed to a hemolysin producing fungus. That is, the claims are directed to a method for using hemolysins to monitor environmental exposures to hemolysin producing fungi in the

environment. There is nothing in any of the art cited that teaches or suggests using fungal hemolysins to monitor exposures to fungi.

Many scientists have tried to develop assays for monitoring indoor fungi, using one of two approaches. They have either tried to quantify a low molecular weight toxin produced by a given fungus, or then have tried to find a surface protein on the conidia or mycelia that was unique. Both of these approaches have failed.

In the first approach, the low molecular weight toxins, *e.g.*, trichothecenes, are metabolized too quickly in most cases to measure exposure in a meaningful way (17). Since antibodies are not produced against these toxins, simpler procedures such as ELISA says are not possible. Also, most low molecular weight toxins are produced by more than one fungus, so that an assay for the toxin does not necessarily identify the specific fungus producing the toxin. In the case of surface proteins, their presence is highly dependent on the substrate on which they grow. Therefore, surface proteins are unreliable for use in assaying exposure to fungi.

Claims 3-5 and 19-21 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim

the subject matter which applicant regards as the invention.

This rejection is respectfully traversed. Claim 21 has been amended to correct a self-evident typographical error: "homiletical active fractions" should be --hemolytic active fractions--. "Active fragments thereof" has been clearly defined above, as well as in the specification, to mean those fragments that have the same activity as the isolated hemolysin and thus can be used to determine if a mammal has been exposed to a hemolysin producing fungus. Claim 3 claims contacting the sample with labeled antibodies and detecting the label. It is clear English that the label detected is that used to label the antibodies. There is no recitation of "synthetic medium" in claim 21, and the Examiner's rejection is not understood.

Art Rejections

Claims 3-5 are rejected under 35 U.S.C. 102(b) as being anticipated by Ishida et al., *Annual Report of Tohoku College of Pharmacy* No. 39, pp. 189-194, 1992.

This rejection is respectfully traversed. There is nothing at all in Ishida et al. that would lead one to use fungal hemolysins to monitor for environmental exposures to fungi in an indoor environment. Ishida et al. specifically referred to the "pathogenicity" of

Aspergillus fumigatus and the role of asp-hemolysin in the pathogenic process, as shown by Ishida et al.'s further publications cited herein, (4, 5, 14, 15). It is clear that Ishida et al. were solely interest in asp-hemolysin for its role as a virulence factor in the infection process by which *A. fumigatus* causes infectious disease. In addition, Ishida et al. described the use of asp-hemolysin in the potential pharmaceutical application of cholesterol control. Ishida et al. give no indications that asp-hemolysin can be used for environmental exposure analysis, which is the centerpiece of the present application.

The claims of the present application are directed to determining if a mammal has been exposed to a hemolysin-producing fungus without specifically claiming any specific assay method for detecting the hemolytic protein. ELISA is merely one method used to detect and quantify these proteins, and the present invention is not limited to the use of ELISA.

For the first time, the present inventors have taught that fungal hemolysins can be used to create assays to evaluate environmental exposures. In this case, the fungal hemolysins are used as quantifiable "biomarkers" of exposure to these fungi. The present claims are not limited to a specific detection method,

rather, the claims are directed to the use of hemolysin as a protein that can be monitored to quantify exposure. The hemolysin can be found in an environmental sample with fungi in it, or in a bodily fluid such as blood, urine, etc. of the person or animal exposed.

Claims 19-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Ishida et al. in view of Fukuchi et al., *Biological Pharmacy Bulletin* vol. 19, No. 4, pp. 547-550, 1996.

This rejection is respectfully traversed. There is nothing in Ishida et al. that suggests determining if a mammal has been exposed to a hemolysin producing fungus. Ishida et al. merely disclose that *Aspergillus* hemolysin could be determined using a chemiluminescence assay. Fukuchi et al. add nothing to Ishida et al. with respect to determining if a mammal has been exposed to a hemolysin producing fungus, because Fukuchi et al. merely disclose that *Aspergillus* hemolysin can be isolated and purified. No combination of Ishida et al. and Fukuchi et al. would lead one skilled in the art to use a method for purifying and detecting fungal hemolysin to the presently claimed invention. Once again, it is emphasized that merely detecting fungal hemolysin is not the invention. The invention claimed herein is a method for determining exposure to a

hemolysin producing fungus by analyzing for the fungal hemolysin. This is neither taught nor suggested by any of the cited art.

In view of the above, it is respectfully submitted that the claims are now in condition for allowance, and favorable action thereon is earnestly solicited.

Respectfully submitted,

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19 ~~20.~~ (Amended) A method for determining if a mammal has been exposed to a hemolysin-producing fungus comprising contacting a sample from said mammal with labeled antibodies to fungal hemolysin or active fragments thereof and detecting the label to determine the presence of antigens to fungal hemolysin, wherein the fungal hemolysin is isolated by culturing a strain of fungus ~~**on a synthetic medium**~~, removing cells and debris from the culture to recover supernatant, and isolating ~~**homiletical**~~ **hemolytically** active fractions of fungal hemolysin.